Notes on ....

Powdery bark in *Eucalyptus accedens* deters arthropods? An evaluation using ants

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Abstract. Powderbark wandoo (*Eucalyptus accedens*) has a powdery triterpenoid-containing substance on the surface of its smooth bark, which is formed from sloughing peridermal cells. When compared with the similar-appearing wandoo (*E. wandoo*), which occurs in the same area and which does not accumulate powder, fewer bark-associated arthropods are found. Exposure to this powder accelerated mortality of the ant, *Iridomyrmex chasei*, a species that tends scale and other sap-sucking insects on the foliage of eucalypts. Ants of this and two other species were unable to reach baits on the top of vertical wooden dowels that had been coated with powder taken from the bark of *E. accedens*. The powder may deter arthropods from living or moving on the bark by chemical or physical means. It is postulated that the function of the powder is to reduce the threat from herbivorous or scale-tending arthropods that may live on, or traverse, the bark of this species.

Keywords: Tree trunk, invertebrate, *Eucalyptus* forest, powder, *Eucalyptus accedens*.

Introduction

Bark provides a wide range of refugia for invertebrates and represents an important source of nutrition to such animals, either directly, or through the fungi, lichens, algae and microorganisms that live on and within it. It also acts as a resting place for invertebrates that move within or across the area in which the trees occur. As part of a larger study of the fauna associated with trunks of Australian eucalypts, we recently examined the arthropod fauna associated with wandoo (*Eucalyptus wandoo*) and powderbark wandoo (*E. accedens*) in the Western Australian wheatbelt (Majer *et al.* 2002, 2003).

Both species possess exfoliating bark and have smooth, light-coloured trunks that are similar in appearance. Powderbark wandoo differs from wandoo in having a bark that is covered in a powdery substance, from which it receives its name. Majer *et al.* (2002, 2003) quantified the species richness and general abundance of various arthropod orders on the bark of these two tree species. They found that powderbark wandoo, when compared with wandoo, yielded generally lower numbers of arthropods (6301 *vs* 7754 individuals in bark traps on 20 trees) and fewer species (422 species from 140 families *vs* 497 species from 159 families in bark plus intercept traps on 20 trees). These differences were significant for many of the invertebrate orders.

Measurements of nutrient content of live and dead bark of the two tree species indicated some significant differences (Majer *et al.* 2003), but these differences were not consistent across elements, suggesting that nutrient levels are not a major determinant of the trends in arthropod levels on the two tree species. Our observations also indicate that there are no large differences in the bark roughness, presence of fissures, or general heterogeneity of bark microhabitats between the two species (see photos in Majer *et al.* 2002, 2003). It therefore appears that nutrient levels or gross bark texture is not related to the differences in arthropod assemblages between the two tree species.

There are examples in the literature of leaves, fruits or stems of plants being covered with microscopic crystals of epicuticular wax, which may protect the plant against herbivorous invertebrates (Stork 1980; Edwards 1982; Eigenbrode 1996; Juniper 1995) or Hemiptera-tending ants (Haberlandt 1909). In view of the similarity in appearance of the trunks of the two eucalypt species in our study, and also the fact that they grow at the same sites, we postulated that the powder on the bark of powderbark wandoo contributed to the observed differences in arthropod abundance and richness. In this note, we report on the origin and nature of the powder of powderbark wandoo and the possible role of the powder in limiting arthropod use of the trunk of the tree.

Methods

The study was undertaken in wandoo/powderbark woodland at Dryandra (32° 56'S, 117° 11'E), southwest Australia, approximately 160 km south-east of Perth. Trees were distributed throughout a 20 ha section of the administrative compartment known as Peters Block; powderbark wandoo trees tended to be on the upper half, while wandoo trees tended to be on the lower half of the landscape. Except where indicated, all measurements and samples were taken at 1.5 m above the ground. It was not possible to measure live bark because it merged in with other living tissue of the trunk. However, both species decorticate bark, so the thickness of 10 samples of freshly-fallen bark beneath each tree was measured with a micrometer.

Powder was collected from powderbark wandoo trees using a clean paint brush by brushing the trunk in a downwards motion into a plastic food container. This material was stored in a refrigerator at 4°C. Sub-samples were plated on malt agar to see if there were any associated microorganisms. These were identified by a local specialist. A Liebermann-Burchard test (Brieskorn and Herrig 1959) was performed on the powder to test for the presence of triterpenoids. Slivers of phelloderm of both species were also collected for sectioning and microscopic examination.

To examine the impact of powder on arthropods, five filter paper discs were dipped in bark powder, and the surplus was shaken off to leave a film of powder of similar thickness to that on the trunks. These, and five clean filter papers, were individually placed in petri dishes. A drop of water was added to maintain moisture. Five worker ants (*Iridomyrmex chasei*) were then placed in each petri dish and the lids were replaced. Dishes were maintained in darkness at 20°C, with a drop of water added daily, and observed at 12 h intervals to record the numbers of live and dead animals. This experiment was conducted at Curtin University using locally-collected ants. However, *I. chasei* also occurs at Dryandra and is known to ascend the trunks of various tree species, where it tends sap-sucking insects (Hemiptera).

A further experiment was performed in the vicinity of Curtin University in which 20 cm long, 13 mm diameter wooden dowels were lightly dusted with powder from the powderbark wandoo trees and the upper end was coated with dilute honey bait. Ten powdered and 10 powder-free dowels were then 'planted' vertically in the ground and continuously observed over a 24 h period. Ants that climbed the dowels to reach the honey were scored and collected for subsequent identification. This experiment was performed on 5–6 April 2003 and again on 1–2 May 2003; the second experiment differed from the first in that the dowels were placed at an angle of 45° from the vertical in order to make them easier to climb.

Results

Powderbark wandoo had a much thinner exfoliated bark than wandoo (mean ± SD: 0.28 ± 0.20 *vs* 1.16 ± 0.45 mm). Examination of the powderbark wandoo bark surface under a stereo-microscope revealed a coating of peridermal cells that were sloughing off, either in clumps or singly. Examination of the powder that had been brushed from the trunk indicated that this comprised of sloughed-off peridermal cells, with powder grains measuring on average 35 µm in length. The cells appeared to be empty, although some contained structures that may be fungal hyphae. Plating of the sub-sample of powder revealed the presence of a range of common saprophytic, epiphytic fungi (*Scytalidium* sp., *Aspergillus* sp., *Ulocladium* sp., *Aureobasidium* sp.), whose presence would be expected on decaying organic matter. The chemical analysis of the powder confirmed the presence of triterpenoids. Wandoo bark was similar in

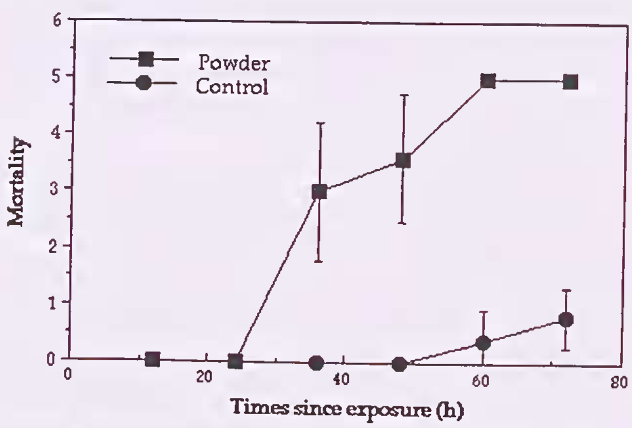

Figure 1. Mean mortality (number of ants per dish ± SD) of *Iridomyrmex chasei* ants (n = 5 replicates of 5 ants per dish) exposed to discs of filter paper that have, or have not, been coated with powder from the bark of powderbark wandoo trees.

appearance and also exhibited sloughing peridermal cells, which did not accumulate to form a powder.

Ant mortality was much greater on the powdered paper discs than on the control discs (Fig. 1), with all ants on the powdered discs having died after 72 h, compared with only 1 death per disc in the controls. Inspection of dead ants indicated that the powder had adhered to their body surfaces, with the greatest concentration around the mouthparts and on the tarsi.

All ant species that ascended the dowels were common species that also occurred at Dryandra. In the first baiting experiment, single individuals of *I. chasei*, *Pheidole ampla perthenesis* and *Paratrechina longicornis* ascended the clean dowels to reach the bait; although several ants attempted to climb the powdered dowels, only one ant (*I. chasei*) ascended a powdered dowel, and this ant fell off after climbing half the distance. In the second experiment, in which dowels were set at a 45° angle, ants reached the honey bait on seven of the 10 clean dowels (mean ± SD: 2.5 ± 2.2 ants), but no ants successfully ascended those that had been powdered; all ants were *P. longicornis*.

Discussion

Our findings suggest that the powder of powderbark wandoo trunks may be responsible for the lower usage of trunks by arthropods, when compared with the similar appearing, and co-occurring wandoo tree species.

The powder of powderbark wandoo trunks may influence arthropods in a number of ways. The triterpenoids or other unknown chemicals in the powder may have a repellent or toxicity effect against arthropods. Repellency is a possibility that we did not test directly, although it is noteworthy that the powder was odourless to humans. Our bioassay with *I. chasei* shows that the powder is capable of causing direct insect mortality, which could have been caused by the toxic triterpenoids or other chemicals in the powder. Evidence against this is the fact that ants attempted to climb the dowels, even when powder was present. Toxic or repellent effects of chemicals in the powder could be tested in the future by exhaustive solvent extraction of the powder before conducting boassays.

Secondly, the powder may have blocked the spiracles of certain arthropods, hence interfering with their normal respiration. Close inspection revealed that this did not happen, as the powder grains are at least double the diameter of the spiracles of *I. chasei*, which are of a size that is typical of many bark-associated arthropods.

Thirdly, the powder may render the bark difficult to walk on. This is supported by the concentrations of powder that had accumulated on the tarsi of ants which were exposed to this material, and also the fact that few or no ants were able to climb the powdered dowels. A similar mechanism has been reported in the pitcher plant, *Sarracenia purpurea*, in which wax inside the pitcher sloughs off when insects attempt to climb out, causing them to fall into the fluid within (Juniper 1986). Wax blooms on the stems of various species of *Macaranga* have also been found to prevent ants from walking across them (Federle *et al.* 1997), and wax layers on the surface of juvenile *Eucalyptus nitens* and *E. globulus* prevent chrysomelid beetles from adhering to their surface (Edwards 1982).

The final possibility is that the powder is simply a physical nuisance to the normal movement and activity of animals to which it adheres. The accumulation of powder around the mouthparts of the *I. chasei* we studied suggests that they tried to remove the powder from their bodies. Possibly this diverted their attention or energy for climbing the trunks of *E. accedens*. Whatever the explanation, our bioassay with dowels shows that the powder effectively prevents ants from climbing the dowels, and thus can deter ants and potentially other arthropods from climbing the trunks.

There may, of course, be other reasons why this tree species accumulates powder on the surface of its bark. Our data in Majer *et al.* (2002) indicate that the invertebrates that are apparently deterred from walking on the powderbark wandoo trunks represent several feeding guilds. Importantly, ants and sap-sucking hemipterans are noticeably less diverse on the powderbark wandoo than the wandoo (43 *vs* 54, and 9 *vs* 15 species respectively in bark traps) (Majer *et al.* 2002). The powder does not totally exclude trunk-associated arthropods but, by reducing the abundance of bark-feeding species on the trunk, and by making it more difficult for folivores to reach the canopy of the tree (Proctor *et al.* 2002), the powder on the bark of *E. accedens* may reduce the overall levels of herbivory and associated injury to the tree.

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References

Brieskorn H and Herrig H 1959 The chemical mechanism of the Liebermann-Burchard color reaction in steroids, terpenes, and their esters. Archiv für Pharmacologie 292: 485–496.

Edwards PB 1982 Do waxes on juvenile *Eucalyptus* leaves provide protection from grazing insects? Australian Journal of Ecology 7: 347–352.

Eigenbrode SD 1996 Plant surface waxes and insect behaviour. In: Plant Cuticles – An Integrated Functional Approach (ed G Kerstiens) Bios, Oxford, 201–222.

Federle W, Maschwitz U, Fiala B, Riederer M and Hölldober B 1997 Slippery ant-plants and skilful climbers: selection and protection of specific ant partners by epicuticular wax blooms in *Macaranga* (Euphorbiaceae). Oecologia 112: 217–224.

Haberlandt G 1909 Physiologische Pflanzenanatomie. Wilhelm Engelmann, Leipzig.

Juniper BE 1986 The path to plant carnivory. In: Insects and the Plant Surface (eds BE Juniper, TRE. Southwood) Edward Arnold, London, 195–218.

Juniper BE 1995 Waxes on plant surfaces and their interactions with insects. In: Waxes: Chemistry, Molecular Biology and Functions (ed RJ Hamilton) Oily, West Ferry, Dundee, 157–174.

Majer JD, Recher HF, Heterick BE and Postle AC 2002 The canopy, bark, soil and litter invertebrate fauna of the Darling Plateau and adjacent woodland near Perth, Western Australia, with reference to the diversity of forest and woodland invertebrates. Pacific Conservation Biology 7: 229–239.

Majer JD, Recher HF, Graham R and Gupta R 2003 Trunk invertebrate faunas of Western Australian forests and woodlands: Influence of tree species and season. Austral Ecology 28: 629–641.

Proctor HC, Montgomery KM, Rosen KE and Kitching RL 2002 Are tree trunks habitats or highways? A comparison of oribatid mite assemblages from hoop-pine bark and litter. Australian Journal of Entomology 41: 294–9.

Stork NE 1980 Role of wax blooms in preventing attachment to brassicas by the mustard beetle, *Phaedon cochleariae*. Entomologica Experimentalis et Applicata 28: 100–107.